Qualitative and Quantitative Control of Adult Hemoglobin Synthesis—A Multiple Allele Hypothesis[1]

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THE existence of two forms of human hemoglobin, adult and fetal, has long been recognized. Fetal hemoglobin (hemoglobin-*f*) is the preponderant form in the erythrocytes of the fetus and the newborn infant. Beginning in early prenatal life, adult hemoglobin is produced in increasing proportion until, by the end of the first year of postnatal life in the majority of individuals, it completely replaces fetal hemoglobin. In some individuals with chronic anemia, either inherited or acquired, a hemoglobin apparently identical with the normal fetal type (Sansone and Cusmano, 1950; Liquori, 1951; Singer, et al., 1951; Rich, 1952; Itano, 1952; Goodman and Campbell, 1952) may persist.

In addition to the normal fetal and normal adult types, abnormal molecular species of human hemoglobin have been identified in recent years. They appear to be different forms of adult hemoglobin. A hypothesis, that a series of multiple alleles affects the synthesis of the adult hemoglobins, is developed in the ensuing discussion.

The erythrocytes of certain individuals assume crescent-shaped and multipointed configurations when deprived of oxygen. This property, called sickling, is associated in some cases with a chronic hemolytic anemia known as sickle cell anemia or sickle cell disease. In the more prevalent condition known as sickle cell trait or sicklemia, the presence of sickling is not associated with hemolytic anemia. An early study of the inheritance of sickling (Taliaferro and Huck, 1923) led to the conclusion that a dominant allele is responsible for the transmission of this erythrocyte property, but no genetic distinction was made between sickle cell anemia and sickle cell trait. More recently (Beet, 1949; Neel, 1949), it has been postulated that individuals with sickle cell trait are

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heterozygous, while those with sickle cell anemia are homozygous, for the sickle cell allele. This holds in the majority of cases of sickle cell disease, but it is now evident that in rare instances a single allele for sickling, when it is combined with a different genetic aberration, produces a disease similar in its manifestations to sickle cell anemia. Four distinct inherited bases for chronic anemia in which sickling cells are present have been described. These will all be included under the term sickle cell disease in this discussion. Only the modification which results from homozygosity for the sickle cell allele will be called sickle cell anemia.

Normal adult hemoglobin (hemoglobin-*a*) is the only form distinguishable in the great majority of non-anemic adults. Both hemoglobin-*a* and sickle cell hemoglobin (hemoglobin-*b*) are present in sickle cell trait erythrocytes (Pauling et al., 1949). Matings of normal individuals with those having sickle cell trait result on the average in equal numbers of children of these two types. Among the progeny of numerous matings of two sickle cell trait individuals, approximately one-fourth of the children have sickle cell anemia, in which the only form of adult hemoglobin is hemoglobin-*b;* one-half have sickle cell trait (hemoglobins *a* and *b*), and one-fourth have only hemoglobin-*a* (Neel, 1951). Hemoglobin-*a* and a second abnormal form of adult hemoglobin, hemoglobin-*c* (Itano and Neel, 1950), are present in non-anemic individuals having the condition called hemoglobin-*c* trait. The children resulting from the mating of a sickle cell trait individual with a hemoglobin-*c* trait individual may be of four types. These are normal, sickle cell trait, hemoglobin-*c* trait, and the modification of sickle cell disease called sickle cell-hemoglobin-*c* disease, in which the two abnormal forms in the parents (*b* and *c*) are both present, and hemoglobin-*a* is absent. A third abnormal hemoglobin, hemoglobin-*d* (Itano, 1951) has been detected in one family. One parent had sickle cell trait and the other hemoglobin-*d* trait, in which hemoglobins-*a* and *d* are present. Two of the children had hemoglobin-*d* trait and two had sickle cell-hemoglobin-*d* disease, characterized by the simultaneous presence of hemoglobins-*b* and *d*.

A fourth form of sickle cell disease evidently results from double heterozygosity in the sickle cell and thalassemia genes and has been observed in matings of individuals with sickle cell trait and thalassemia minor (Silvestroni and Bianco, 1952).

Sickling has been observed only in erythrocytes which contain hemoglobin-*b*. In the various forms of sickle cell disease, a significant correlation has been observed between the degree of anemia and the relative amount of hemoglobin-*b* present (Itano, 1952).

The sickling test is a satisfactory qualitative test for the presence of hemoglobin-*b*, and has been used in studies of the inheritance of the sickling phenomenon. However, electrophoretic analyses and solubility determinations

are necessary to distinguish conclusively the different forms of sickle cell disease as well as to detect abnormal hemoglobins in non-sickling erythrocytes. Electrophoretic analyses also furnish quantitative information on the proportions of the different hemoglobins in a given specimen.

Hemoglobin-*b* separates from hemoglobin-*a* on electrophoretic analysis. Both of these hemoglobins are observed upon analysis of sickle cell trait hemoglobin. In sickle cell anemia hemoglobin, hemoglobin-*b*, together with a small proportion of hemoglobin-*f*, is observed, while hemoglobin-*a* is absent. The qualitative results of electrophoresis are, therefore, in accord with the heterozygous-homozygous theory for the inheritance of sickle cell trait and sickle cell anemia. However, the quantitative studies on the hemoglobins from 42 genetically unrelated individuals (Wells and Itano, 1951) demonstrated the presence of a wide variation in the relative amounts of hemoglobins *a* and *b* in sickle cell trait. The ratio of the hemoglobins remains constant in a given individual with change in time. The effects of age, sex, some environmental factors, and heredity were examined, and the first three factors were found to have no appreciable influence on the ratio of the two hemoglobins in a person with sickle cell trait. In addition to the 42 unrelated subjects, one family of four individuals with sickle cell trait was examined, and the two children were found to have the same ratio of hemoglobins as one of the parents. Subsequently this ratio was investigated in seven families in which sickle cell trait was detected (Neel, et al., 1951). In some of these families the ratio was constant; in others the ratio differed among different individuals in a family. The genetic mechanism for this variability was not clear.

Additional data on the inheritance of hemoglobin ratios became available with the discovery of families in which both hemoglogins *b* and *c* were present (Itano and Neel, 1950; Kaplan, et al., 1951). The electrophoretic data on the members of these families again suggested the existence of a genetic control of hemoglobin ratios.

RESULTS OF FAMILIAL STUDIES

All of the available data on familial studies of hemoglobin ratios are given in Table 1. The ratios were determined by electrophoretic analyses in the Tiselius apparatus. The experimental conditions, reproducibility, and constancy of the ratio in a given individual have been previously discussed (Wells and Itano, 1951). Family Mc is here reported for the first time. The rest of the data have been derived from previous investigations (Wells and Itano, 1951; Itano and Neel, 1950; Neel, et al., 1951). Direct ratios (a/b, a/c, and b/c) rather than percentages are given in order to facilitate analysis of the data. Figure 1 shows the frequencies of these ratios in the families under study. The ratios 1.4 and 1.9, which correspond respectively to 42 and 34 percent sickle cell hemoglobin, occur most frequently. A similar bimodality was observed among 42

TABLE 1. FAMILIAL ELECTROPHORETIC DATA ON HEMOGLOBIN RATIOS

FAMILY	REF.*	Hb PRESENT AND RATIOS		RATIOS IN CHILDREN WITH 2 FORMS ADULT Hb†		
				S.C. Trait	Hb-*c* Trait	S.C.-Hb-*c* Disease
		Father	Mother	*a/b*	*a/c*	*a/c*
Pe	1	*a/b*, 1.9	*a/b*, 1.4	1.5, 1.5		
Sn	2	*a/b*, 1.4	*a* only	1.4, 1.3, 1.4, 1.3		
Wi_1	2	*a* only	*a/b*, 1.4	2.1, 2.2, 1.9, 2.2		
Wa	2	*a/b*, 1.9	*a* only	1.5, 1.4, 1.4, 1.5, 1.9, 1.9, 2.1, 1.9		
Hi	2	*a* only	*a/b*, 1.9	1.9, 1.8		
Li	2	*a* only	*a/b*, 2.6	1.9, 1.8, 3.5		
St	2	*a/b*, 1.7	*a* only	1.3		
Bo	2	*a/b*, 1.2	*a* only	1.3, 1.4, 1.2, 1.3		
Ca	3	*a/c*, 1.8	*a/b*, 2.0		2.0	0.8
Wi_2‡	3	*a/c*, 2.3	*a/b*, 2.2			0.9, 1.0
Mc	—	*a/b*, 2.0	*a/c*, 2.9	2.9		1.0

* (1) Wells and Itano, 1951; (2) Itano and Neel, 1950; (3) Neel, et al., 1951.

† See Table 2 for data on other children.

‡ The mother in family Wi_2 is one of the children in family Wi_1.

Key to Table 1

a, Normal adult hemoglobin — Hb, Hemoglobin

b, Sickle cell hemoglobin — S.C., Sickle cell

c, Hemoglobin-*c*

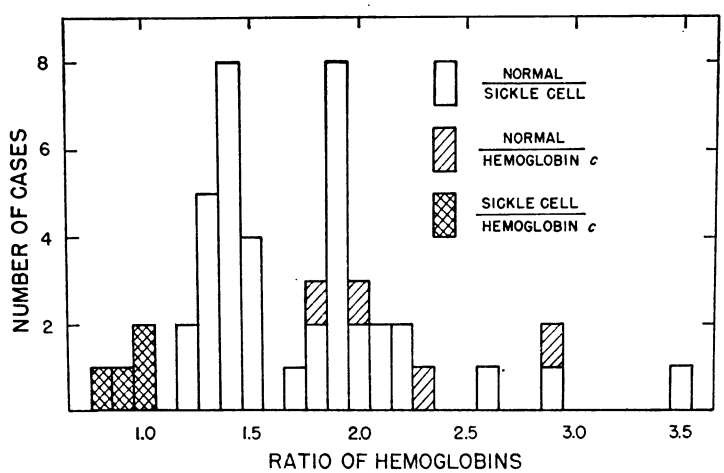

FIG. 1. Frequency distribution of adult hemoglobin ratios in the eleven families included in the present study.

unrelated individuals. Ratios approximating 3 occur in two of the families. With one exception, the ratio of hemoglobin-*b* to hemoglobin-*c* is 0.9 or 1.0. The individual in whom the ratio is 0.8 has a third component, which was

originally reported to be normal adult hemoglobin. Recent studies indicate that this third component is fetal hemoglobin (Itano, 1952).

DISCUSSION

The Allelic Control of Adult Hemoglobin Abnormality

As a point of departure, we can recognize the existence of three genotypes with regard to a single pair of alleles. Letting *sk* represent the normal allele, and *Sk* the sickle cell allele, the three genotypes are *sksk* (normal), *skSk* (sickle cell trait), and *SkSk* (sickle cell anemia). The absence of hemoglobin-*a* in sickle cell anemia suggests that the *Sk* allele controls a complete absence or modification of an essential step in the biochemical differentiation of this form. The question now arises: how are the genetic bases of the other abnormal adult hemoglobins (*c* and *d*) related to the *sk* locus?

According to the results of electrophoretic analyses, the sickle cell allele diverts less than half the total adult hemoglobin production in sickle cell trait to the net synthesis of an abnormal hemoglobin. While making no assumptions for the present as to the genetic control of hemoglobin-*c* synthesis, we may note that in hemoglobin-*c* trait, the observed proportion of hemoglobin-*a* has always been higher than that of hemoglobin-*c* (Table 1). In sickle cell-hemoglobin-*c* disease, the two abnormal hemoglobins, *b* and *c*, are present in nearly equal proportions, and hemoglobin-*a* is absent.

In discussing the relationship of inheritance studies to these biochemical observations, let us first assume that the locus of the gene which is responsible for the abnormal nature of hemoglobin-*c* is different from the *sk* locus and consider the implications of this assumption. The hemoglobin-*c* allele may be designated as *C* and its normal allele as *c*. Accordingly, in families Ca, Wi_2, and Mc, the results of the mating, *Sksk cc* × *sksk Cc* have been observed. Four phenotypes have been recognized among the children, and these would presumably be of the following genotypes: *sksk cc* (normal), *Sksk cc* (sickle cell trait), *sksk Cc* (hemoglobin-*c* trait), and *Sksk Cc* (sickle cell-hemoglobin-*c* disease). According to this genetic analysis, a normal pathway for hemoglobin synthesis may be available in sickle cell-hemoglobin-*c* disease. However, hemoglobin-*a* is absent, although the electrophoretic results cited above suggest that the net effect of *sk* or *c*, acting independently, is greater than that of their respective aberrant alleles, *Sk* or *C*. It is difficult on the basis of these observations to postulate a biochemical mechanism whereby the genotype *skSk cC* would result in the complete absence of hemoglobin-*a*. The most plausible alternative postulate is that *Sk* and *C* are allelic; i.e., no normal allele is present at the *sk* locus in this disease.

We shall therefore postulate that the control of the net synthesis of hemoglobins *a*, *b*, and *c* resides in allelic genes. Each individual receives one member

of this multiple allelic series from each of his parents. Investigations of the sickling properties of sickle cell trait erythrocytes (Sherman, 1940) indicate that in all probability each erythrocyte contains two hemoglobins, *a* and *b*. This allelic series, therefore, acts on a cellular basis, displaying a direct relationship between the genetic constitution of the cell and the type of hemoglobin synthesis it is able to perform. In addition, each individual possesses a separately controlled mechanism for the synthesis of fetal hemoglobin which is latent in most adults but which may be activated in chronic anemias.

The Allelic Control of Rate of Hemoglobin Synthesis

In every specimen of sickle cell trait hemoglobin which has been examined electrophoretically, the percentage of hemoglobin-*a* has been higher than that of hemoglobin-*b*. The mean corpuscular hemoglobin (MCH) of sickle cell trait erythrocytes is normal, so that if the total capacity of the hemoglobin-*b* synthetic mechanism were that represented by its contribution in sickle cell trait, the MCH of sickle cell anemia erythrocytes would be low. Actually the MCH is normal or higher than normal in sickle cell anemia; even if the fetal hemoglobin which is found in sickle cell anemia is taken into account, the amount of hemoglobin-*b* per cell is in the majority of cases more than twice that found in sickle cell trait. A similar phenomenon has been noted in sickle cell-hemoglobin-*c* disease.

The postulate of allelic determination of adult hemoglobin types implies that the relative amounts of two forms of adult hemoglobin in a given individual represent the net result of hemoglobin production by two simultaneous processes. The ratios in sickle cell trait erythrocytes indicate that the net rate of synthesis of hemoglobin-*a* averaged over the entire period of hemoglobination of an erythrocyte, is always higher than that of hemoglobin-*b*. But the relative rates of normal and aberrant hemoglobin synthesis differ among individuals with sickle cell trait. The normal MCH of sickle cell anemia erythrocytes suggests a longer than normal period of synthetic activity by a mechanism which produces an abnormal hemoglobin at a lower than normal rate. The observed variations in the hemoglobin ratios in sickle cell trait may result from the existence of rate modifications in the net synthesis of either or both of the adult hemoglobins present.

Consideration of the data in sickle cell-hemoglobin-*c* disease is of value in deciding whether the hemoglobin-*b* mechanism has more than one rate modification. In contrast to sickle cell trait, this disease is characterized by a hemoglobin ratio which varies but slightly among individuals. In eight individuals from seven different families, the ratio of hemoglobin-*b* to hemoglobin-*c* lies in the narrow range 0.8 to 1.0 (Itano, 1952). The most probable explanation for the relative constancy of this ratio is that only one characteristic rate is associated with the synthesis of each of these hemoglobins, so that whenever

they occur together, their ratio is the same. The assumption of more than one rate modification for one mechanism would necessitate the same assumption for the other; and we should also have to assume that in the individuals examined to date, the corresponding rate modifications of the sickle cell and hemoglobin *c* mechanisms have always occurred together. The multi-modality of the hemoglobin ratios (Figure 1) and the lack of more than two of the modal ratios in any given family of sickle cell trait individuals (Table 1) suggest the presence of a relatively simple genetic control of these ratios.

We shall, therefore, postulate that the variations in the hemoglobin ratios in sickle cell trait are the result of genetically controlled modifications in the rate of synthesis of hemoglobin-*a*, but not of hemoglobin-*b*. This postulate leads to the simplest genetic hypothesis consistent with the data, namely that the inherited synthetic mechanisms for the synthesis of hemoglobin-*b* and hemoglobin-*c* have only one characteristic rate apiece, which are nearly equal, and that the normal mechanism exists in the population as three genetic modifications which produce hemoglobin-*a* at relative rates 1.4, 1.9, and 3 times that of the hemoglobin-*b* mechanism. Of the 47 ratios shown in Figure 1, only 5 deviate more than 15 percent from the assigned ratios, and the maximum deviation is 21 percent. As far as the available data are concerned, we may consider that the hemoglobin-*b* mechanism, the hemoglobin-*c* mechanism, and the three rate modifications of the hemoglobin-*a* mechanism depend on alleles. The individual members of the families considered in this study have been classified according to their postulated genotypes in Table 2. It may be seen that the assumption of multiple allelism does not result in any inconsistencies between the hypothesis and the data.

Genetically this assumption implies that there is a locus at which the occurrence of a mutation may result either in the formation of an abnormal molecular form of hemoglobin or merely in the alteration of the rate of formation of normal adult hemoglobin. According to this hypothesis the ratios in the sickling offspring of the mating of a normal and a sickling individual are determined solely by the genetic constitution of the non-sickling parent. Such an individual receives the *Sk* allele from his affected parent, and one of three alleles governing normal adult hemoglobin synthesis at a particular relative rate from his normal parent. The presence of two, but no more than two, ratios among the sickling children is readily explained on this basis; other explanation as, for example, independent "modifying factors" (Neel, et al., 1951) would lead to different results, less readily compatible with the genetic data as they exist. The data are by no means conclusive, however.

Terminology

The symbol *Sk* has heretofore been employed for the allele responsible for the formation of sickle cell hemoglobin and *sk* for its normal alternative (Neel, et

al., 1951). This terminology will be retained with the necessary modifications to specify each of the five postulated alleles. Sk^b and Sk^c will represent the alleles which result in hemoglobin-*b* and hemoglobin-*c* respectively. The three rate-characterized alleles for hemoglobin-*a* may be designated as $sk^{1.4}$, $sk^{1.9}$, and sk^3. An allele, $sk^{2.2}$, would provide better agreement with the data for families W_1 and Wi_2; other intermediate ratios have been observed among unrelated individuals. More extensive familial studies must be conducted before we can determine whether normal alleles other than the three postulated ones exist in the population.

The assumption of three alleles which result in the formation of hemoglobin-*a* at different rates suggests that an individual who is of the normal phenotype

TABLE 2. POSTULATED GENOTYPES DERIVED FROM FAMILIAL ELECTROPHORETIC DATA

FAMILY	PARENTAL COMBINATION (FATHER × MOTHER)	NUMBER OF CHILDREN									
		S.C. Trait			Hb-*c* Trait			S.C.-Hb-*c*. Disease Sk^bSk^c	Normal† *sk sk*	S.C. Anemia Sk^bSk^b	Not Tested
		$sk^{1.4}Sk^b$	$sk^{1.9}Sk^b$	sk^3Sk^b	$sk^{1.4}Sk^c$	$sk^{1.9}Sk^c$	sk^3Sk^c				
Pe	$sk^{1.9}Sk^b$ × $sk^{1.4}Sk^b$	2	0	0	0	0	0	0	0	1	0
Sn	$sk^{1.4}Sk^b$ × $sk^{1.4}sk^{(1.4)}$*	4	0	0	0	0	0	0	4	0	1
Wi_1	$sk^{1.9}sk^{(1.9)}$* × $sk^{1.4}Sk^b$	0	4	0	0	0	0	0	1	0	3
Wa	$sk^{1.9}Sk^b$ × $sk^{1.4}sk^{1.9}$*	4	4	0	0	0	0	0	5	0	1
Hi	$sk^{1.9}sk^{(\ \ )}$* × $sk^{1.9}Sk^b$	0	2	0	0	0	0	0	6	0	1
Li	$sk^{1.9}sk^3$* × sk^3Sk^b	0	2	1	0	0	0	0	4	0	0
St	$sk^{1.9}Sk^b$ × $sk^{1.4}sk^{(\ \ )}$*	1	0	0	0	0	0	0	4	0	2
Bo	$sk^{1.4}Sk^b$ × $sk^{1.4}sk^{(1.4)}$ *	4	0	0	0	0	0	0	2	0	0
Ca	$sk^{1.9}Sk^c$ × $sk^{1.9}Sk^b$	0	0	0	0	1	0	1	1	0	0
Wi_2	$sk^{1.9}Sk^c$ × $sk^{1.9}Sk^b$	0	0	0	0	0	0	2	1	0	0
Mc	$sk^{1.9}Sk^b$ × sk^3Sk^c	0	0	1	0	0	0	1	0	0	1

* Probable parental genotype deduced from those of children.

† *sksk* includes all of normal genotypes.

may be one of six genotypes which differ in the rate at which his erythrocytes are hemoglobinated. These genotypes may be distinguished by examining the hemoglobin ratios in the offspring resulting from matings with individuals who have the Sk^b or Sk^c allele. This situation is somewhat analogous to the situation presented by three wild-type iso-alleles of *Drosophila melanogaster* at the *ci* locus (Stern and Schaeffer, 1943). Each of these alleles produces in the homozygous condition the same normal wing venation at the usual culturing temperature of 25–26° C., and special tests are required to distinguish these alleles. Iso-alleles have been defined as alleles indistinguishable except by special tests, and the three alleles of normal adult hemoglobin conform to this definition.

The families in Table 1 and 2 were selected on the basis of the presence of the sickle cell allele, and the parents may be assumed to have among them a random selection of the normal iso-alleles. Eliminating the duplication due to the relationship between Wi_1 and Wi_2, and counting only the genes, the presence of which have been established with certainty, we obtain 8, 12, and 3, respectively, for the incidence of $sk^{1.4}$, $sk^{1.9}$, and sk^{3}, among the unrelated parents. Analysis of the data on 42 unrelated individuals reveals that 16 have ratios of 1.4 or 1.5, and 15 have ratios between 1.7 and 2.0, inclusive. The presence of these modal values has previously been noted. If we consider the ratios in all 42 individuals, we find that all but two of these fall within $\pm$ 15 percent of 1.4, 1.9, and 3, and the maximum deviation is 20 percent. The numbers of individuals in these groups are 22, 16, and 4, respectively. The two independent samplings are, therefore, in rough agreement as to the relative frequencies of the postulated iso-alleles.

Relationship of the Other Human Hemoglobins

We have not discussed the relationship to our hypothesis of the fetal hemoglobin which is present in the erythrocytes of sickle cell disease and other chronic anemias. The production of this form of hemoglobin is probably governed by a mechanism genetically different from the adult hemoglobin mechanisms. Its identification and significance in sickle cell disease have been discussed elsewhere (Itano, 1952). Hemoglobin-*d* has been found in only one family, and one of the parents was not available for hemoglobin studies. Furthermore, this hemoglobin does not separate electrophoretically from sickle cell hemoglobin; its identification depends on solubility determinations. Until a method for the determination of the ratio of sickle cell hemoglobin to hemoglobin-*d* is found, quantitative analysis of the inheritance of this hemoglobin is not possible. In the one family studied, the allele for hemoglobin-*d* behaves qualitatively as a member of the multiple allelic series here postulated.

The Inheritance of Thalassemia

It is of interest to speculate upon the relationship of our hypothesis to the findings in thalassemia, an inherited anemia which apparently results from a different type of abnormality in hemoglobin metabolism. No abnormal hemoglobin is known to be associated with thalassemia, but varying amounts of fetal hemoglobin are present, together with normal adult hemoglobin. The MCH is subnormal, and the primary effect of the thalassemia gene appears to be on the synthesis of normal adult hemoglobin (Rich, 1952). The block in the adult mechanism apparently results in the compensatory continuance of the fetal mechanism. In spite of a marked erythroid hyperplasia of the bone marrow and a relatively mild hemolytic process, a severe anemia and peripheral erythroblastosis are present in thalassemia major. These observations imply

that although there is an increased demand by the peripheral blood for erythrocytes, their rates of maturation and release from the hemopoietic tissues are abnormally low. The low MCH values indicate that the retention of the fetal mechanism and the lengthened time of hemoglobination do not completely compensate for the reduced rate of adult hemoglobin synthesis. In thalassemia minor, similar findings are present to a milder degree. Anemia is mild or absent, and a polycythemia may be present as an apparent compensatory response to the low MCH. It has been postulated that the thalassemia major results from homozygosity in the thalassemia gene and that thalassemia minor results from heterozygosity (Valentine and Neel, 1944).

Although the difference between the major and minor forms is usually pronounced, gradations in the severity of each have been noted, and some "mild" and "moderate" cases have been described (Smith, 1948). The multiple allele hypothesis for the rate of synthesis of normal adult hemoglobin provides a possible explanation for the diversified findings in thalassemia. The thalassemia gene apparently is non-allelic with the sickle cell locus (Silvestroni and Bianco, 1952). The familial, as well as individual, differences which have been observed in the manifestations of thalassemia may be due to differences in the effectiveness of the different genotypes for normal adult hemoglobin synthesis described in this paper, in combination with the independent block to normal hemoglobin synthesis which constitutes the net effect of the thalassemia gene.

In sickle cell-thalassemia, which apparently is due to double heterozygosity in the sickle cell and thalassemia genes (Silvestroni and Bianco, 1952), both normal adult and sickle cell hemoglobins are present, but in contrast to sickle cell trait, the percentage of normal hemoglobin is relatively low (Sturgeon, et al., 1952; Itano, 1952). Although the MCH is low, the average amount of sickle cell hemoglobin per erythrocyte is higher than the corresponding values in sickle cell trait and sickle cell-hemoglobin-*c* disease and greater than half that in sickle cell anemia, suggesting that the thalassemia gene does not impair the rate of synthesis of sickle cell hemoglobin. The resulting preponderance of sickle cell hemoglobin probably is the principal biochemical factor in causing the hemolytic anemia.

CONCLUSION

The hypothesis outlined above has been presented with the realization that although it is consistent with all available data, the accumulation of additional information may require its modification. However, we believe that its presentation at this time is of value for several reasons. First, it introduces the concept of relative rates of synthesis as the determining factor in hemoglobin ratios. Second, it postulates that the ratio of the two inherited forms of adult hemoglobin in an individual with sickle cell trait results from variation in the normal

rather than the sickle cell hemoglobin producing mechanism. This suggests that, contrary to an earlier opinion (Neel, et al., 1951) studies of families in which both parents sickle should be as valuable as those in which only one parent sickles in determining the genetic basis of this quantitative variation. Finally, the number of families available to any one laboratory for study is limited, and the provision of a working hypothesis such as we have furnished might stimulate the collection of critical test data in other laboratories.[4]

SUMMARY

A hypothesis has been introduced which attributes the hemoglobin ratios in erythrocytes containing more than one form of adult hemoglobin to differences in the average net rates of synthesis of the hemoglobins. Differences in the hemoglobin ratios among individuals of a given phenotype (e.g., sickle cell trait) may result from the existence of at least three rate modifications of the mechanism for the synthesis of normal hemoglobin. The simplest genetic hypothesis which is in accord with the available familial data on hemoglobin ratios is that the sickle cell hemoglobin mechanism, the hemoglobin *c* mechanism, and the three rate modifications of the normal hemoglobin mechanism depend on alleles.

The possible applicability of this hypothesis in explaining the presence of hemolytic disease in sickle cell-thalassemia heterozygotes and the variability of the clinical and hematologic findings in thalassemia has been discussed.

Electrophoretic data have been presented on a previously unreported family in which both sickle cell hemoglobin and hemoglobin *c* have been found.

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[4] Since this manuscript was submitted for publication, the author has studied a large family in which hemoglobins *a*, *b*, and *c* are present. The preliminary data for this family are consistent with the multiple allele hypothesis for the control of hemoglobin type, but not of ratios. The results, which will be published in a separate paper, suggest that interactions other than those considered in the present paper may influence the relative rates of hemoglobin synthesis.

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